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THE EFFECTS OF ERYTHROCYTE INTERMEDIATES ON OXYGEN DISSOCIATION--ETC(U)
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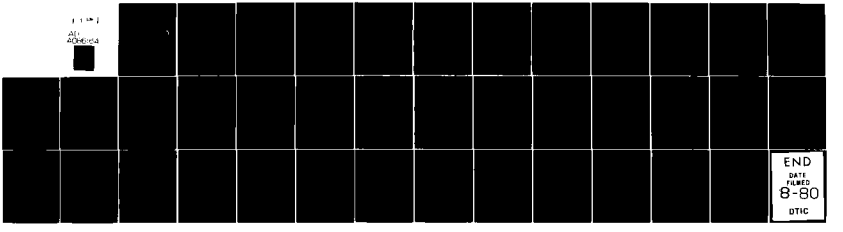

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The Effects of Erythrocyte Intermediates on Oxygen Dissociation Properties of Hemoglobin

FINAL REPORT

GEORGE J. BREWER

MAY 1979

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DADA17-69-C-9103

Departments of Human Genetics and Internal Medicine
The University of Michigan
Ann Arbor, Michigan 48109

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER / 2. GOVT ACCESSION NO. AD-A086 164	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) THE EFFECTS OF ERYTHROCYTE INTERMEDIATES ON OXYGEN DISSOCIATION PROPERTIES OF HEMOGLOBIN	5. TYPE OF REPORT & PERIOD COVERED Final March 1969 - December 1974
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) George J. Brewer	8. CONTRACT OR GRANT NUMBER(s) DADA17-69-C-9103
9. PERFORMING ORGANIZATION NAME AND ADDRESS Depts. of Human Genetics & Internal Medicine The University of Michigan Ann Arbor, Michigan 48109	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62110A.3A062110A827.00.007
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research & Development Command Fort Detrick Frederick, Maryland 21701	12. REPORT DATE May 1979
	13. NUMBER OF PAGES 39
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)	15. SECURITY CLASS. (of this report) Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Altitude adaptation, erythrocyte, 2,3-diphosphoglycerate, oxygen affinity, hypoxia, acute mountain sickness, altitude polycythemia, altitude preadaptation with drugs.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The biochemical mechanisms by which the erythrocyte elevates its 2,3-diphosphoglycerate and decreases its oxygen affinity were studied. It appears that a complex set of mechanisms involving the rate limiting enzymes of glycolysis, hexokinase, phosphofructokinase, and pyruvate kinase, are involved

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Secondly, an approach to preadaptation to altitude was attempted using pharmacological means. Orally administered phosphate was used in a double-blind study involving 20 subjects taken to Pikes Peak (altitude 4300 m). While differences were not large, the phosphate-treated group had better overall performance in a number of areas compared to the placebo group. Some differences reach statistical significance. These results suggest that a drug with greater effect on reducing oxygen affinity than phosphate might have very salutory effects in altitude preadaptation.

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I. GENERAL INTRODUCTION

Our work under this contract resulted in development of knowledge in two general areas. The first concerns the biochemical mechanisms of human red blood cell metabolic adaptation to altitude. The second concerns a pharmacological approach to preadapting the human red cell to altitude. We will take up these two topics in sequence.

II. BIOCHEMICAL MECHANISMS OF RED BLOOD CELL 2,3-DIPHOSPHOGLYCERATE INCREASE AT HIGH ALTITUDE

A. INTRODUCTION

Components of the oxygen transport system respond to high altitude in ways which help compensate for the reduced partial pressure of oxygen. The red blood cell responds metabolically with a build-up of 2,3-diphosphoglycerate (2,3-DPG) levels. Increased 2,3-DPG levels decrease hemoglobin-oxygen affinity, or shift the oxygen dissociation curve rightward, which can be expected to augment tissue oxygen delivery if arterial oxygen saturation remains high.

Our purpose was to identify the biochemical mechanisms that were most likely responsible for the previously reported increase in 2,3-DPG levels. Studies were conducted 1) under baseline conditions and during short-term (8-10 days), high-altitude exposure to 2300 m at Climax, Colorado and to 4300 m on Pikes Peak, Colorado, 2) among normal residents of Leadville, Colorado (3100 m), 3) among residents of Leadville with chronic mountain polycythemia, and 4) for comparative purposes, among residents of 240 m at Ann Arbor, Michigan. Possible biochemical mechanisms were identified by first identifying through statistical techniques the glycolytic intermediates on which the variation in 2,3-DPG levels was dependent. Then the positions of these intermediates in the glycolytic pathway and the changes in their mean levels after high-altitude exposure were inspected. Inferences were then drawn concerning the enzymatic mechanisms most likely responsible for elevating 2,3-DPG levels. These inferences are based upon both the data of this paper and the prior knowledge of which enzymes are rate limiting in human red cell glycolysis. The examination of biochemical mechanisms under both short-term and long-term experimental conditions and method of data analysis employed distinguishes this effort from previous reports on the mechanisms of 2,3-DPG increase at high altitude.

B. MATERIALS AND METHODS

1. Study Groups

a. Short-term studies (Climax and Pikes Peak)

Five male and five female adult laboratory personnel from Ann Arbor, Michigan (240 m) were brought to the 3400-m elevation of Climax, Colorado and remained there for 8 days. Male-female mean sex differences in this sample (and the Pikes Peak study) were removed by simple linear regression. Blood samples were drawn before ascent in Ann Arbor and after 6 hours, 2 days, 4 days, 6 days, and 8 days in Climax.

Seven U.S. Army enlisted men and three female laboratory personnel were housed on top of Pikes Peak, elevation 4300 m, for 10 days. All subjects were residents of Denver, Colorado (1600 m) except one who had come from a lower altitude (240 m). Blood samples were drawn before ascent in Denver and then after 6 hours, 2 days, 4 days, 7 days, and 10 days on Pikes Peak.

b. Long-term studies (Leadville and Ann Arbor)

Normal high-altitude residents consisted of twenty-eight males who had lived in Leadville (3100 m) for more than one year. None reported having any difficulty in adjusting to Leadville's altitude and all had hematocrits of less than 55 volumes percent.

Twenty-eight males, also residents of Leadville (3100 m) for at least one year, with chronic mountain polycythemia were chosen from the records of the St. Vincent's Hospital in Leadville. These subjects were identified on the basis of having hematocrits consistently above 55 volumes percent in the absence of phlebotomy.

Forty-one healthy male residents of Ann Arbor (240 m) comprised the low-altitude sample. All had lived in Ann Arbor for at least one year.

2. Study Variables

Blood (20 ml) from the antecubital vein was drawn to measure hematocrit using the capillary tube methods. Well mixed samples (500 ml) were immediately precipitated in trichloracetic acid (TCA), frozen in dry ice and transported to Ann Arbor for determination of 2,3-DPG and adenosine triphosphate (ATP) levels enzymatically. Additional well mixed samples (10 ml) were precipitated immediately in perchloric acid (PCA), frozen in dry ice and transported to Ann Arbor for measuring glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), fructose diphosphate (FDP), dihydroxyacetone phosphate (DHAP), 3-phosphoglycerate (3PG), 2-phosphoglycerate (2PG), phosphoenolpyruvate (PEP), pyruvate (PYR), lactate (LACT), and adenosine diphosphate (ADP) using enzymatic methods of Minakami et al. (1955) as modified by Oelshlegel et al. (1972). Hemoglobin was read colorimetrically from well mixed hemolysates which had been frozen and transported to Ann Arbor.

3. Statistics

Regression analysis was used to assess the relationship between variation in 2,3-DPG levels and the variation in each of the other glycolytic intermediates or cofactors. The coefficient of determination (r^2) was computed for each variable in each study group, and in the short-term studies, at each measurement time. Computations were performed for the glycolytic variables when expressed both in μmoles/gm Hb in μmoles/liter red blood

cell water. Variables whose r^2 values were significant ($P < .05$) for both units of measurement were termed "key variables" insofar as it is variation in these variables that is most closely linked statistically to variation in 2,3-DPG levels.

In the short-term studies, change in the mean levels of the variables over all the measurement times was determined with the Hotelling T^2 test. Comparison of variable means in the Ann Arbor normal with the Leadville normal and Leadville polycythemic samples was made using the two sample (Student's) t-test. The Scheffe correction for multiple comparison was used to avoid the inflation of type I errors incurred by multiple use of the t-test. Mean differences are reported as significant when $P < .05$. Values are reported as mean ± SEM.

C. RESULTS

1. Short-term Studies (Climax and Pikes Peak)

2,3-DPG levels increased during short-term exposure at Climax (3400 m) and Pikes Peak (4300 m) (Figure 1, Tables 1 and 2). Maximal increases of 15% - 20% above baseline values occurred at both altitudes. The rise in 2,3-DPG levels at Pikes Peak was more rapid than at Climax where a transient decrease initially occurred.

Key variables identified in the Climax study were: G6P, FDP, DHAP, 2PG, PEP, and the ratio of ADP to ATP. At Pikes Peak, the key variables were: G6P, 3PG, PEP, and the ratio of ADP to ATP levels. The key variables common to both studies (Figure 2) were ADP/ATP, G6P, and PEP.

Among the key variables, mean levels of ADP/ATP and PEP changed during both short-term studies (Tables 1 and 2). Additional glycolytic variables changing in both studies were FDP and 3PG. LACT at Climax and G6P at Pikes Peak also underwent mean change (Tables 1 and 2).

2. Long-term Studies (Leadville and Ann Arbor)

2,3-DPG levels among Leadville residents with chronic mountain polycythemia were significantly higher than in Ann Arbor residents but Leadville normals' 2,3-DPG levels, while also higher, were not significantly different from Ann Arbor values (Table 3).

Key variables could not be identified in either the Ann Arbor normal or Leadville normal samples due to the absence of significant relationships between 2,3-DPG and other glycolytic variables. In the Leadville polycythemic sample, key variables were G6P, F6P, DHAP, and the ratio of ADP to ATP levels (Figure 3).

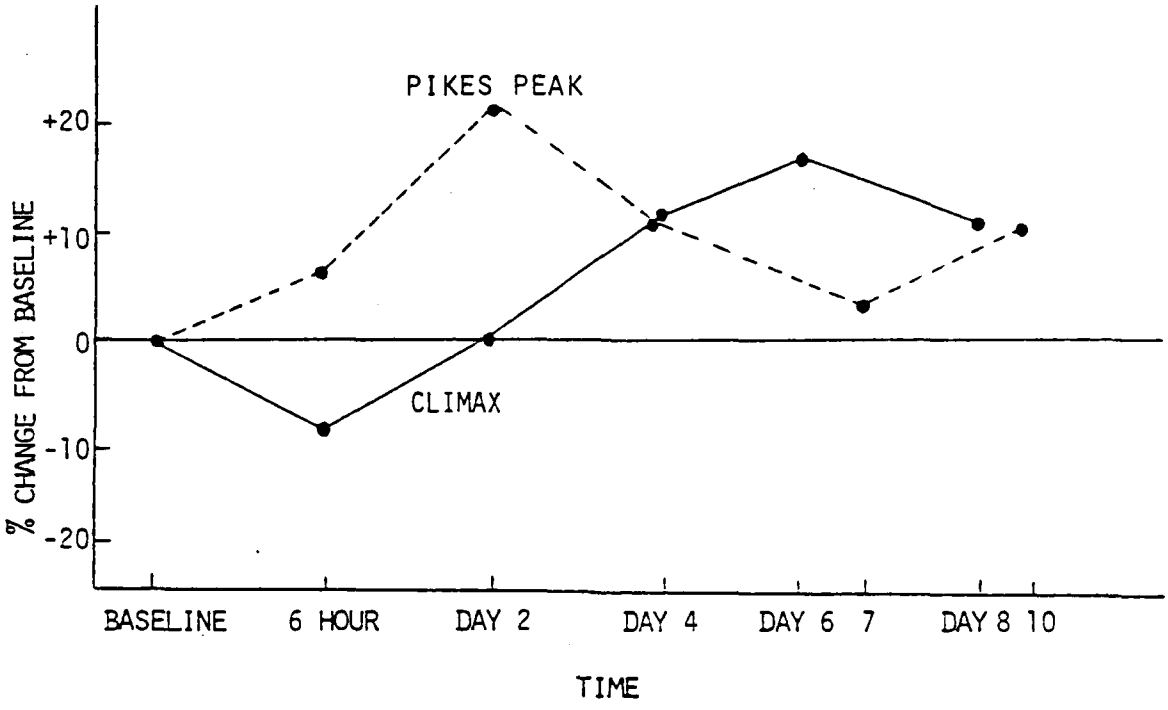

Figure 1. Time course of changes in mean 2,3-DPG levels. Sample sizes = 10 for Pikes Peak (4300 m) and Climax (3400 m) at each measurement time.

TABLE 1

GLYCOLYTIC AND HEMATOLOGIC VARIABLES DURING CLIMAX (3400 m) STUDY (n = 10)

Variable	Ann Arbor Baseline	6 Hours	2 Days	4 Days	6 Days	8 Days	T^2 Test
2,3-DPG[1]	8452	8913	8830	9777	10087	9436	$P < .05$
ADP/ATP Ratio	.112	.132	.111	.133	.112	.113	
Glucose-6-phosphate	45	50	55	52	56	54	$P \geq .05$
Fructose-6-phosphate	14	15	14	13	14	15	
Fructose diphosphate	2.5	3.2	2.2	2.9	1.7	1.4	$P < .05$
Dihydroxyacetone phosphate	15	17	11	12	13	14	
3-phosphoglycerate	111	115	105	103	99	88	$P < .05$
2-phosphoglycerate	11	15	11	13	13	12	
Phosphoenol pyruvate	21	22	19	23	23	18	$P < .05$
Pyruvate μmoles/ℓ w.b.	48	40	78	41	42	46	
Lactate μmoles/ℓ w.b.	894	1084	993	781	680	939	$P < .05$
Hemoglobin g/100 ml	14.8	14.9	15.1	14.8	15.1	15.8	$P < .05$

[1]Variables are expressed in μmoles/ℓ RBC water unless indicated otherwise.

TABLE 2

GLYCOLYTIC AND HEMATOLOGIC VARIABLES DURING PIKES PEAK (4300 m) STUDY (n = 10)

Variable	Denver Baseline	6 Days	2 Days	4 Days	7 Days	10 Days	T^2 Test
2,3-DPG[1]	7830	8304	9413	8839	8013	8326	$P < .05$
ADP/ATP Ratio	.179	.132	.154	.147	.201	.164	$P < .05$
Glucose-6-phosphate	58	55	60	64	60	64	$P < .05$
Fructose-6-phosphate	14	11	12	12	13	12	
Fructose diphosphate	2.0	3.2	4.3	4.0	4.7	4.6	$P < .05$
Dihydroxyacetone phosphate	15	17	20	16	21	17	
3-phosphoglycerate	81	89	98	88	94	93	$P < .05$
2-phosphoglycerate	33	14	10	15	13	12	
Phosphoenol pyruvate	27	25	27	20	29	20	$P < .05$
Pyruvate μmoles/ℓ w.b.	56	58	53	58	47	63	
Lactate μmoles/ℓ w.b.	855	1149	944	1404	930	1408	
Hemoglobin g/100 ml	16.0	16.3	15.9	16.6	16.2	17.4	$P < .05$

[1]Variables are expressed in μmoles/ℓ RBC water unless indicated otherwise.

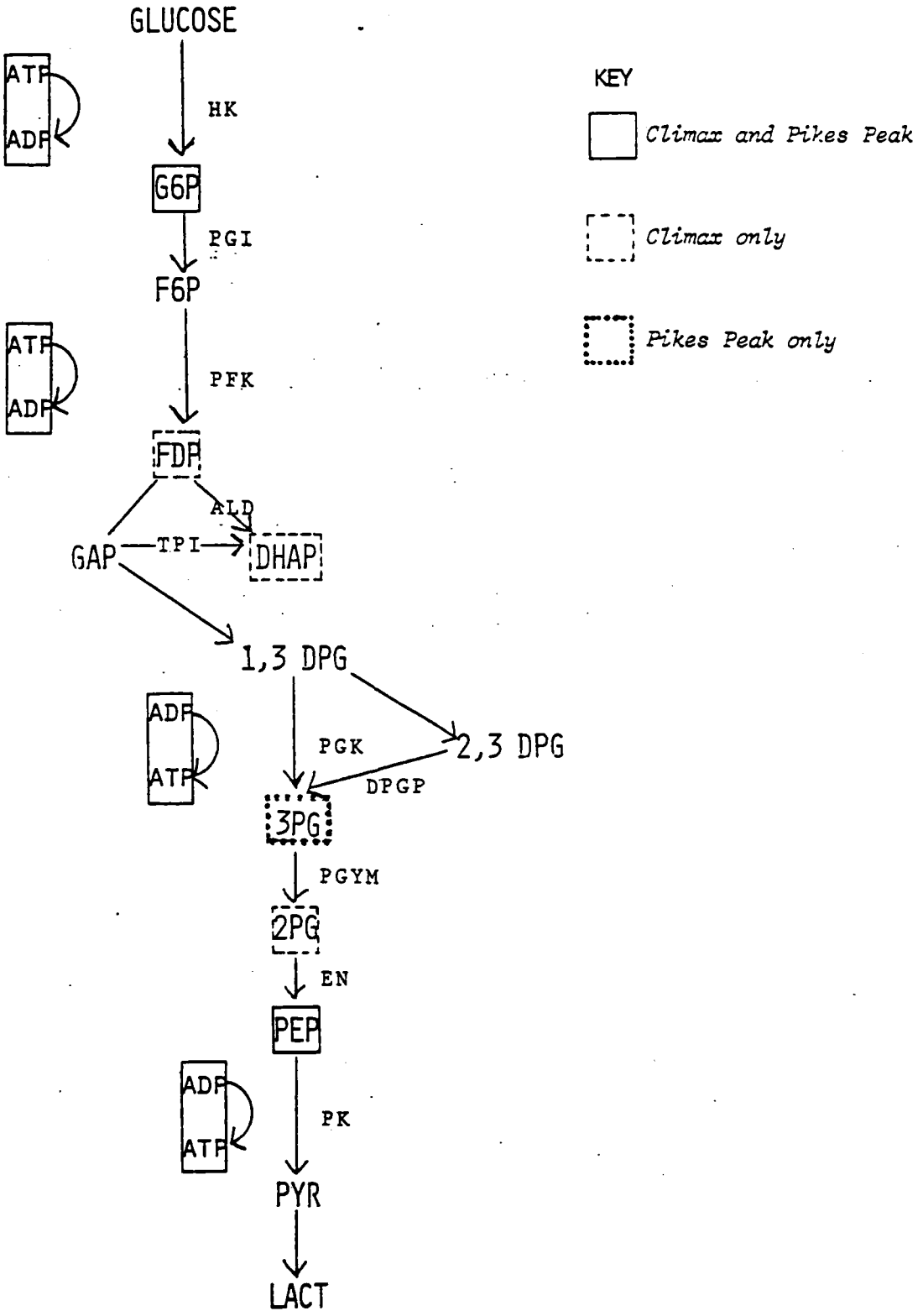

Figure 2. Key variables in short-term studies.

TABLE 3

VARIABLES MEASURED IN GROUPS OF HIGH- (3100 m) AND LOW- (240 m) ALTITUDE RESIDENTS

Variable	(1) Leadville Polycythemics	(2) Leadville Normals	(3) Ann Arbor Normals	t-test (1) vs(3)	t-test (2) vs(3)
2,3-DPG[1]	8823 ± 225	8535 ± 142	8167 ± 122	P < .05	
ADP/ATP Ratio	.110 ±	.127 ±	.120 ±		
Glucose-6-phosphate	58 ± 3	55 ± 2	53 ± 2		
Fructose-6-phosphate	17 ± 1	15 ± 1	12 ± 2		
Fructose di-phosphate	3.0 ± .5	1.8 ± .2	2.5 ± .3		
Dihydroxy-acetone phosphate	22 ± 2	14 ± 2	12 ± 1	P < .05	
3-phosphoglycerate	100 ± 5	104 ± 4	77 ± 6	P < .05	P < .05
2-phosphoglycerate	12 ± 1	12 ± 1	11 ± 2		
Phosphoenol pyruvate	18 ± 1	19 ± 1	23 ± 1	P < .05	P < .05
Pyruvate μmoles/ℓ w.b.	50 ± 5	42 ± 4	46 ± 4		
Lactate μmoles/ℓ w.b.	1284 ± 153	888 ± 52	729 ± 72	P < .05	
Hemoglobin g/100 ml w.b.	19.0 ± .4	17.3 ± .2	15.8 ± .2	P < .05	P < .05

[1]Variables are expressed in μmoles/ℓ RBC water unless indicated otherwise.

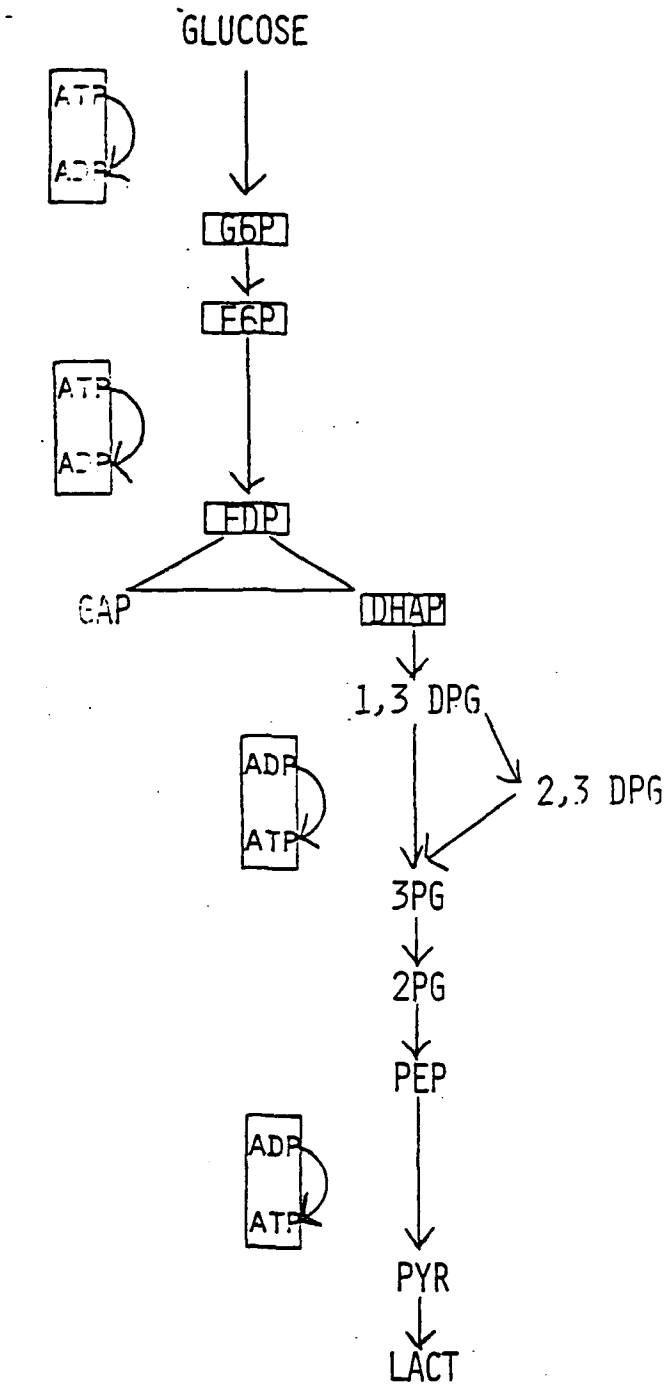

Figure 3. Key variables in Leadville polycythemics. Key variables enclosed in boxes.

DHAP, 3PG, and LACT levels were elevated in the Leadville polycythemic subjects and PEP values lower compared to the Ann Arbor sample. Among these, only DHAP was a key variable. 3PG and PEP were also variables undergoing change in the Leadville normal subjects.

D. DISCUSSION

During altitude exposure, the 2,3-DPG of the human erythrocyte adjusts to the hypoxic exposure by an increase, ranging from 15% to 20% (Figure 1). We presume that the primary stimulus for the increase of erythrocyte 2,3-DPG is hypoxia. The mechanisms by which hypoxia in the body stimulates the elevation of 2,3-DPG in the erythrocyte are not completely clear. One mechanism which probably operates is respiratory alkalosis due to hyperventilation. Alkalosis operates to increase the in vivo activity of phosphofructokinase (PFK) of the erythrocyte. However, this mechanism alone cannot account for the types of glycolytic intermediate patterns which have been observed under various hypoxic conditions. We are not completely certain of the identity of the additional mechanisms, but we suspect that they are hormonal.

Irrespective of the signals transmitted by the body to the erythrocyte to increase 2,3-DPG levels, since 2,3-DPG is a product of glycolysis, changes in 2,3-DPG levels require changes in the glycolytic mechanism. Given this fact, can we establish which enzymes of glycolysis are involved in the 2,3-DPG adjustments of the red cell to hypoxia? Assays of the activities of the red cell enzymes themselves before and during altitude exposure are unlikely to be helpful. Since the circulating mature red cell does not synthesize new protein, the V_{max} of the enzymes will be expected to remain unchanged. What does probably change is the regulation of the rate-limiting enzymes of red cell glycolysis. These enzymes normally operate under partial inhibition. The rate-limiting enzymes are hexokinase (HK), PFK, and pyruvic kinase (PK). We believe our best hope of discovering which of these enzymes are involved in the adjustment of red cell glycolysis to hypoxia lies in studying the glycolytic intermediates of the erythrocyte. The levels of these intermediates at any point in time are a "biopsy" of the in vivo activity of the various enzymes at that time. Previously, interpretation of glycolytic intermediate patterns has relied on the crossover plot method of Chance (1958) in which the levels of intermediates after hypoxic stimulus or in patients with hypoxic disorders were plotted as a percent change from their prehypoxic levels or the levels of a control group of subjects. The limitations of the approach are: (1) that comparisons between a subject's intermediate levels at two times or between two groups of subjects cannot rigorously take account of variation within a group of subjects or variation over multiple times, and (2) that comparisons permitted do not lend themselves to hypothesis testing and the determination of statistical significance.

The present approach has been designed to go beyond the level of description possible with crossover plots to the identification of variables on which variation in 2,3-DPG levels is statistically dependent. To do this,

the r^2 value, or the coefficient of determination, was calculated between 2,3-DPG and the other glycolytic variables and cofactors. These r^2 values were screened in order to identify the variables on which 2,3-DPG levels were significantly dependent, termed the "key variables." The positions of these variables in the glycolytic pathway were then used to identify enzyme steps potentially involved in 2,3-DPG regulation.

Since each key variable involves a minimum of two enzymes (the enzyme producing and the enzyme catabolizing the intermediate), the task remains to determine for each study 1) which enzymes were most likely involved in 2,3-DPG regulation, and 2) whether alterations in in vivo activities appear to have occurred. This requires some measure of subjective assessment. However, without a direct method available for assessing enzyme activity in vivo, we believe that the approach used here moves us somewhat closer to our goal. In addition, we are limited by lacking data on the rate of glucose consumption or flux through the glycolytic pathway either when 2,3-DPG is increasing (short term) or after a new steady state has been reached (long term). Our studies of glucose consumption have not shown consistent or statistically significant changes. Rørth and Nygaard (1972) have stated that glucose consumption is increased during short-term, high-altitude stress but to our knowledge, they have published no actual data. Difficulty in obtaining consistent results may be related to the failure of the stimuli affecting the cell's metabolism during the in vitro incubation required for measuring glucose consumption to be comparable to the stimuli operating on the red cell in vivo. Also, our calculations indicate that changes in flux under these in vivo circumstances may be rather modest (i.e., 1% increase), and still result in the observed 2,3-DPG changes. Current methods would not detect such small changes in flux. Tentatively, we believe that glycolytic flux increases at least during the initial phase of short-term, high-altitude exposure. This belief is based upon the observation that nearly all the glycolytic intermediates increase within the first 6 hours or 2 days of short-term, high-altitude exposure (Table 2). Without an increase in flux, it seems unlikely that intermediates positioned along the whole length of the glycolytic pathway would all increase. Increased flux is also supported by the observation that glucose consumption is increased in another hypoxic condition, anemia, associated with high 2,3-DPG levels.

Figures 2 and 3 are designed to help us concentrate on the key variables identified by the statistical technique, and the position of these variables in the glycolytic pathway. In the short-term studies (Figure 2), G6P, PEP, and the ratio of ADP to ATP were identified as key variables in both studies. Four other variables were identified as key variables in one or the other of the two studies (Figure 2). The identification of the ratio of ADP to ATP as a key variable is not very specific, since these cofactors are involved in all of the four kinase steps of the pathway. However, the identification of G6P and PEP as key variables in both studies suggests important roles for HK and PK in the modulation of glycolysis at altitude. As mentioned above, the three enzymes which are considered to be rate limiting in red cell glycolysis are HK, PFK, and PK. The product of PFK, FDP, as well as the next

intermediate, DHAP, were identified as key variables during the Climax study but not during the Pikes Peak study. Thus, the data at this point are consistent with the involvement of all three rate-limiting enzymes of red cell glycolysis in the modulation of glycolysis for high-altitude adjustment and elevation of 2,3-DPG levels. Two other variables that were identified as key in one or the other of the two studies are 3PG and 2PG. At present we have no particular hypothesis to expain their role.

The key variables identified in Leadville polycythemics, which of course inolves long-term adjustment to altitude and hypoxia, are to a certain extent parallel to those of the short-term studies (compare Figure 2 and Figure 3). Thus, the ratio of ADP to ATP and G6P are again identified. Additionally, F6P, FDP, DHAP, are identified as key variables, while PEP is not so identified. This data suggests that the rate-limiting enzymes at the front of the glycolytic pathway, namely, HK and PFK, are predominant in the long-term modulation that leads to 2,3-DPG elevation in the Leadville polycythemic population.

No key variables were identified in the Leadville normal and the Ann Arbor study groups. Neither were 2,3-DPG levels significantly greater than Ann Arbor values among the sample of normal Leadville residents, although previous studies of the same population have indicated significant increases.

E. SUMMARY AND CONCLUSIONS

We have described high-altitude investigations aimed at uncovering enzymatic mechanisms responsible for the observed increases in 2,3-DPG levels at high altitude. Studies were conducted at Climax (3400 m) and at Pikes Peak (4300 m) on the effects of short-term, high-altitude exposure and on normal excessively polycythemic residents of Leadville (3100 m) in comparison with a sample of Ann Arbor (240 m) residents. Statistical analysis identified the individual variables (key variables) best able to account statistically, for variation in 2,3-DPG levels. Subsequently, mean levels of each key variable common to the Climax and Pikes Peak studies—G6P,PEP, ADP/ATP—indicated possible HK activation and PK inhibition. In addition, key variables unique to the Climax study suggested PFK activation. Key variables identified in the Leadville polycythemic group—G6P, F6P, FDP, DHAP, ADP/ATP—suggested possible roles for PFK and HK activation in the maintenance of high 2,3-DPG levels. The absence of glycolytic key variables among the Leadville normal and Ann Arbor subjects prevented the identification of 2,3-DPG regulatory mechanisms in either of those studies.

III. THE EFFECTS OF A PHARMACOLOGICALLY-INDUCED DECREASE IN HEMOGLOBIN-OXYGEN AFFINITY ON SHORT-TERM ADAPTATION TO 4300 m

A. INTRODUCTION

A continuing debate concerns the importance of decreased hemoglobin-oxygen affinity for adaptation to high altitude. Our purpose was to examine this issue in the context of short-term responses to 4300 m on Pikes Peak. It is known that red cell 2,3-DPG levels increase in response to high altitude within two to three days, resulting in decreased hemoglobin-oxygen affinity. A previous study had shown that oral treatment with phosphate elevated red cell 2,3-DPG levels by 15% - 20% in normal subjects. This provided us with a tool to preadapt the red cell by increasing 2,3-DPG levels prior to ascent.

Our study design was to create a contrast, double-blind, in 2,3-DPG levels and oxygen dissociation curve positions between two groups of subjects. One group of 10 subjects was administered phosphate, sodium bicarbonate, vitamin C to increase 2,3-DPG levels and to right-shift their oxygen dissociation curves before high-altitude ascent. A second group of 10 subjects received placebos. The 2,3-DPG levels of the placebo group were expected to remain normal. Their oxygen dissociation curves were also expected to remain normal or perhaps left-shifted as a result of respiratory alkalosis experienced during the first two or three days of high-altitude exposure. Our hypothesis was that the drug-treated group with high 2,3-DPG levels would adjust better to high altitude than would the placebo treated, low 2,3-DPG subjects. That is, the drug group would be expected to experience less acute mountain sickness (AMS) and have fewer signs of central nervous system (CNS) hypoxia. In addition, if economy in the oxygen dissociation variable of oxygen transport reduces demands on other components, the drug group might also be expected to show less cardiopulmonary response to high altitute.

B. MATERIALS AND METHODS

1. Subjects

Twenty normal male residents of Ann Arbor (240 m) were selected as study subjects after obtaining their informed consent. All subjects were judged as healthy on the basis of a medical history and a physical examination. Subjects ranged from 20 to 42 years of age. Most were undergraduate or graduate students at The University of Michigan.

Subjects were flown from Detroit to Denver in four groups of five subjects. Each group was immediately driven from Denver to the top of Pikes Peak (4300 m) where they stayed for 6 days. Total time in transport from low to high altitude was 5-1/2 hours for all but two subjects who were

delayed en route. Groups were staggered to lessen workloads at any one time for the investigators. Each group was comprised of placebo and drug subjects.

2. Drug and Placebo Treatment

Subjects were divided randomly in double-blind fashion into drug (n = 10) and placebo (n = 10) groups. Phosphate, vitamin C (ascorbate), and sodium bicarbonate were administered in order to stimulate an increase in 2,3-DPG levels. Information on the rationale and efficacy of these agents is published by Moore et al. (1977). Treatment with all three drugs or placebos began 36 hours before ascent. Sodium bicarbonate drug and placebo treatment were taken for the first 9 hours of this 36-hour predeparture period. Phosphate and vitamin C drug and placebo treatments were continued through the third day of residence on top of Pikes Peak.

Phosphate was administered orally, three times daily in doses of 30 umoles of a 1-M solution containing equal amounts of $NaPO_4$ and KPO_4 (pH = 7.450) mixed with 2 - 3 oz of a quinine flavored, sterile solution. Vitamin C was given four times daily as four, 500-mg capsules containing sodium ascorbic acid, and orange flavoring. Sodium bicarbonate was given three times daily in doses of 1.25 m equivalents/kg body weight mixed in 8 oz of quinine flavored solution. The University of Michigan Hospital Pharmacy prepared the phosphate and sodium bicarbonate drugs and Cooper Drug Company (Detroit, Michigan) supplied the vitamin C. Similarly sized and flavored placebos for the phosphate and sodium bicarbonate drugs were also prepared at The University of Michigan Hospital Pharmacy. Cord Laboratories (Detroit, Michigan) made placebos for the vitamin C. The treatment schedules followed by subjects taking placebos were identical to those subjects taking drugs.

3. Variables

Blood samples (30 ml) were taken from the antecubital vein in Ann Arbor for control measurements and after 6, hours, 24 hours, 48 hours, 96 hours, and 144 hours of exposure to 4200 m. Hemoglobin-oxygen dissociation curve position was measured with an automatic curve analyzer (Instrumentation Laboratories DCA) and are reported as standard P50's (pH = 7.4, PCO_2 = 40 mm Hg). Hemoglobin was measured colorimetrically and 2,3-DPG levels were assayed enzymatically. A sample of arterialized venous blood was used to measure whole blood and intracellur pH in Ann Arbor and after 6 hours and 96 hours at 4200 m using previously described methods.

Ventilation, cardiac output, O_2 consumption and CO_2 production were measured at rest and during exercise in Ann Arbor and after 6 hours and 96 hours on Pikes Peak. Exercise workloads averaged 800 kg-m/min in Ann Arbor and 675 kg-m/min in Pikes Peak on a bicycle ergometer. Minute ventilation was measured with a dry gas meter, recording tidal volume and frequency on a

Gilson Polygraph (Model M5p). O_2 tensions were read with a fuel cell O_2 analyzer on samples of expired air collected in a Douglas bag over a 3-minute period for calculating O_2 consumption. CO_2 tensions were read with a Beckman LB-1 on the same expired air sample and used to calculate CO_2 production. Cardiac output was calculated from the Fick equation using the CO_2 rebreathing technique described by Ferguson and co-workers (1968), to determine venous CO_2 content.

CNS hypoxia was assessed by the subjects' performance on visual and cognitive tasks. Dark adaptation were measured according to previously described methods. Critical flicker fusion was measured as described by Simonson and Brozek (1952). The cognitive tests used were the Neisser letter search, Stroop color reading, digit span memory, nonsense syllable memory, addition, grammatical reasoning, Fitts tapping, digit symbol substitution, and Shephard letter rotation. Details describing their composition and administration are reported by Rose (1973). Visual and cognitive tests were conducted twice in Ann Arbor for baseline measurements and after 6 hours, 24 hours (day 1), and 96 hours (day 4) on Pikes Peak. As further control for interpreting the cognitive tests, an additional group of 10 normal subjects remained in Ann Arbor and were given the cognitive tests following the same protocol as the Pikes Peak subjects.

Incidence of AMS was measured by the General High-Altitude Questionnaire (GHAQ). Subjects filled out the questionnaire at the same time in the morning and evening on three consecutive days in Ann Arbor for control measurements and daily while at high altitude. The incidence of AMS was also evaluated qualitatively as "sick," "well," or "in-between," by a blinded physician (GJB) during the first 48 hours of high-altitude exposure.

C. RESULTS

1. Hematological Variables

The drug-treated group evidenced higher 2,3-DPG levels when compared to the placebo-treated group after 24 hours of high-altitude exposure (Figure 4) ($P < .05$). Drug group 2,3-DPG levels tended to be higher than placebo group levels after the initiation of treatment prior to ascent and after 6 hours on Pikes Peak but these differences were not statistically significant.

Oxygen dissociation curve positions were the same in the drug and placebo groups before treatment prior to ascent (P_{50} = 25.6 ± mm Hg). After 6 hours of high-altitude exposure, the drug-treated group was right-shifted compared to the placebo group (Figure 5, 1-tailed $P < .01$). Curve positions were the same in both groups after 4 days on Pikes Peak. Whole blood and intracellular pH were the same in the two groups at each measurement time.

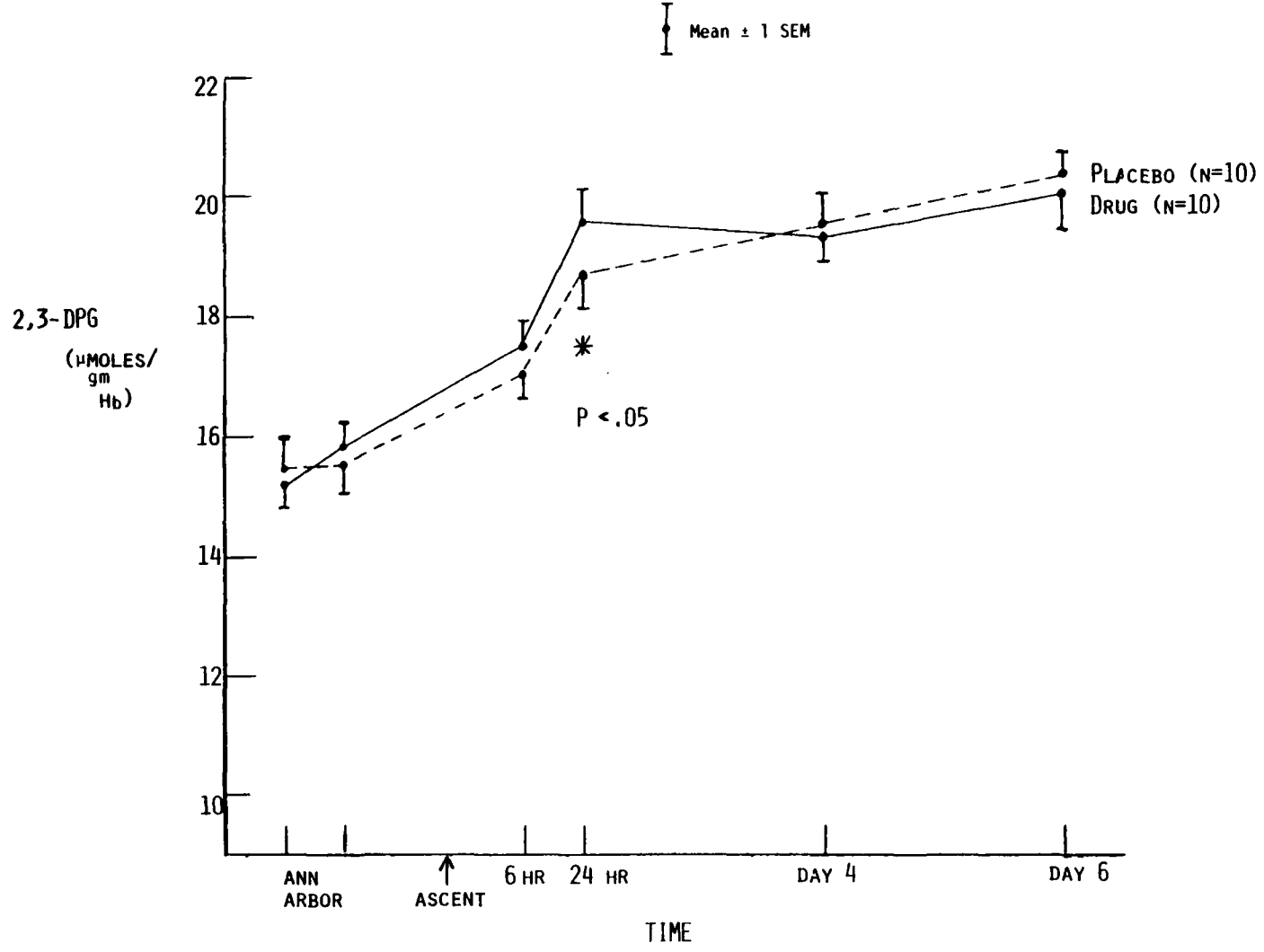

Figure 4. Changes in 2,3-DPG before and after ascent to Pikes Peak.

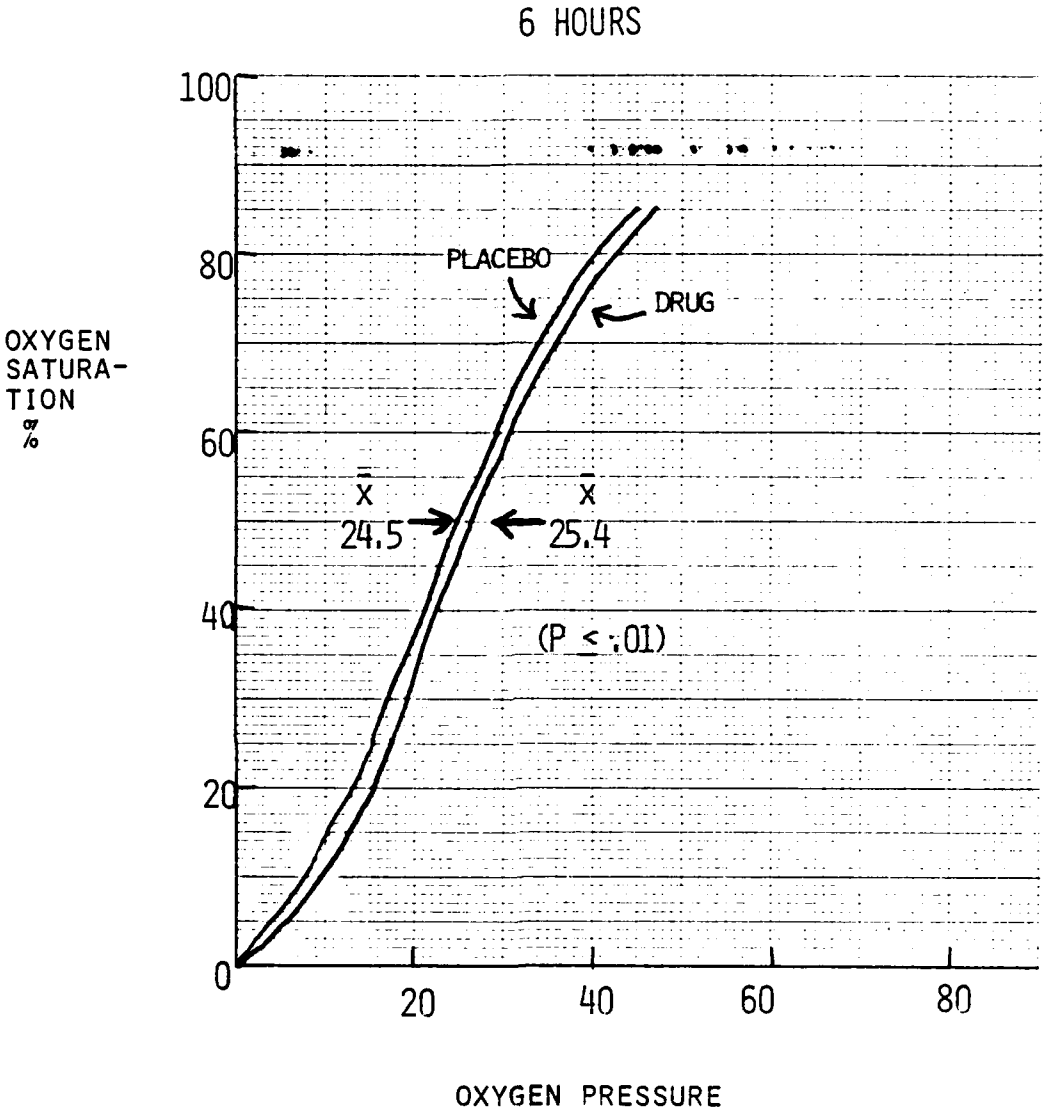

Figure 5. Drug-placebo curve positions (Pikes Peak) (corrected to pH 7.4).

Erythropoietin levels rose after high-altitude ascent in both treatment groups (Figure 6), in keeping with the known stimulatory effect of hypoxia on red blood cell production. Both groups evidence great variability. Thus, while values are not statistically different, the drug-treated group tends to have lower erythropoietin levels than the placebo subjects after 6 and 24 hours of high-altitude exposure.

2. Physiological Variables

The drug and placebo groups evidenced the same O_2 consumption, CO_2 production, ventilation, and cardiac output during rest at low and high altitudes (Table 4). Only CO_2 production was increased during exercise in the drug group on day 4 at Pikes Peak (1-tailed $P < .05$). Alveolar O_2 and CO_2 tensions were not different in the drug and placebo groups during rest or exercise at each measurement time (Table 4).

3. Symptomatology and Performance Variables

Drug-treated subjects evidenced milder symptoms of AMS than did the placebo-treated subjects. Most drug-treated subjects were well whereas most placebo-treated subjects were sick (Table 5). One drug-treated and two placebo-treated subjects were classified as "in-between." The GHAQ also indicated that fewer AMS symptoms developed in the drug-treated compared to the placebo-treated group (Figure 7). The six scales—arousal, irritable, headache, mood, fatigue, and cardiorespiratory—represent three to six correlated items each on the questionnaire as determined in previous applications. Each scale is scored from 1 to 5, representing increasing degrees of severity. The median change for each scale from Ann Arbor to after 6 hours on Pikes Peak demonstrates that the drug group evidences less change than does the placebo group for five of the six scales and the same degree of change in the remaining (irritable) scale. GHAQ scales at subsequent time periods gradually returned to Ann Arbor values and showed no consistent differences between the drug- and placebo-treatment groups.

The dark adaptation test measures the threshold at which light can be perceived at 1-minute intervals after retinal bleaching on a scale of 1 (lightness) to 12 (darkness) such that a higher score means that light was perceived at darker levels of illumination. The relative improvement of Pikes Peak over Ann Arbor levels is attributed to a practice effect. Performance of the drug-treated subjects was better than that of the placebo-treated subjects after 6 hours of high altitude (Table 6). Performance on the critical flicker fusion test was the same in both groups at each measurement time (Table 6).

Results from the tests of digit span memory, letter search, grammatical reasoning, digit symbol substitution, and addition tests showed a

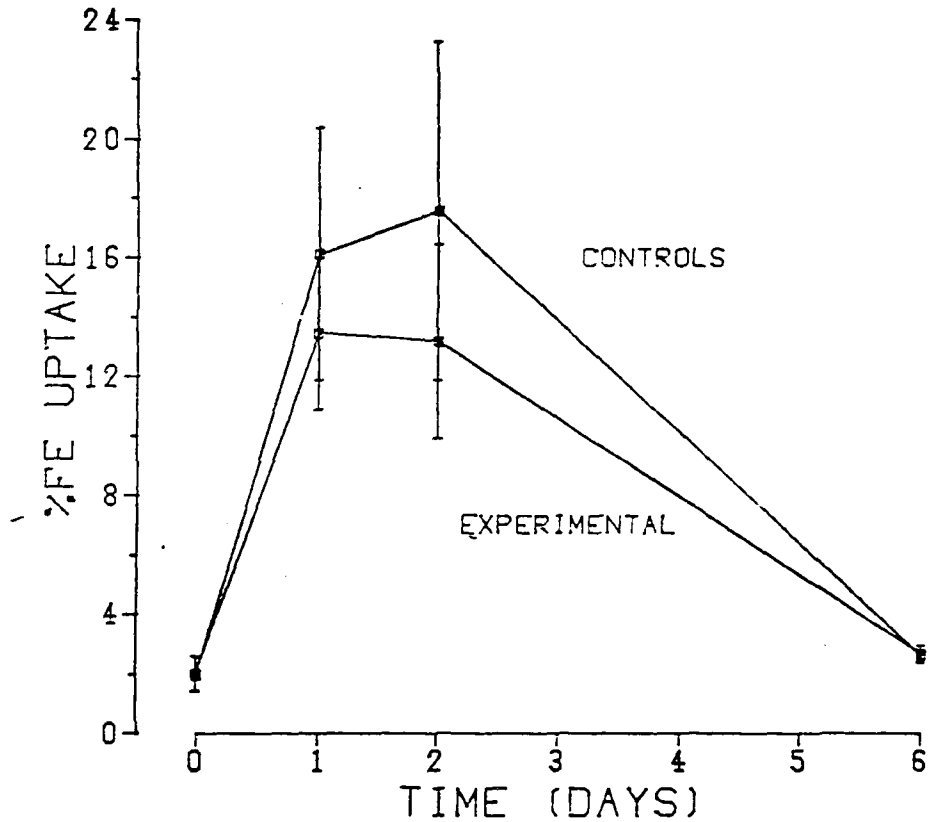

Figure 6. Erythropoietin assays.

TABLE 4

BLOOD GAS MEASUREMENTS

	Ann Arbor		5 Hours, Pikes Peak		Day 4, Pikes Peak	
	Drug	Placebo	Drug	Placebo	Drug	Placebo
pH, whole blood	7.351 ± .007*	7.364 ± .007	7.381 ± .014	7.370 ± .012	7.363 ± .018	7.400 ± .006
pH, intracellular	7.244 ± .006	7.242 ± .005	7.254 ± .011	7.254 ± .0007	7.238 ± .013	7.218 ± .013
Rest:						
P_AO_2	101.7 ± 3.5	95.8 ± 3.4	45.6 ± 1.4	46.6 ± 1.8	48.5 ± 1.1	50.9 ± 2.4
P_ACO_2	33.9 ± 1.7	35.7 ± 1.4	33.6 ± 1.2	32.9 ± 1.3	29.8 ± 1.3	29.6 ± 1.5
CO						
VO_2						
VCO_2						
Exercise:						
P_AO_2	100.3 ± 1.6	98.6 ± 2.5	50.2 ± 0.8	49.1 ± 1.1	53.8 ± 0.7	53.6 ± 0.8
P_ACO_2	39.1 ± 1.2	39.7 ± 1.2	33.2 ± 1.1	32.7 ± 1.6	28.5 ± 1.2	30.0 ± 1.0
CO						
VO_2						
VCO_2						

*Mean ± S.E.M.

TABLE 5

CLINICAL EVALUATION

(Double-Blinded)

	SICK	WELL
DRUG-TREATED SUBJECTS	3	6
PLACEBO-TREATED SUBJECTS	5	3

x^2 not significant

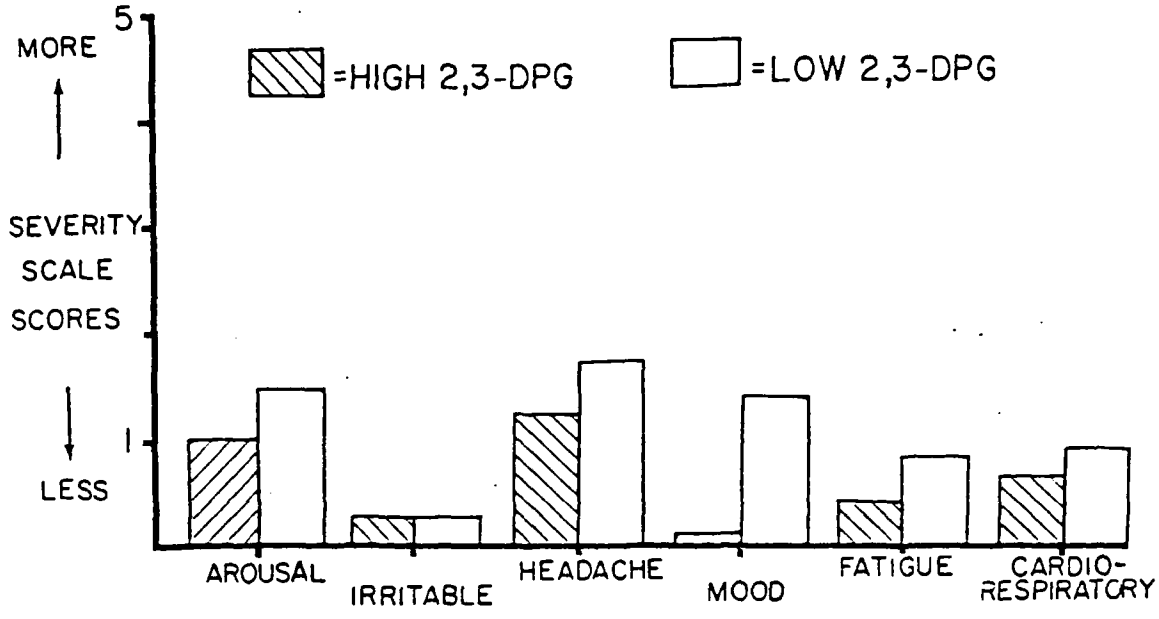

Figure 7. Symptomatology scores.

TABLE 6

VISUAL TESTS

	Critical Flicker Fusion	Dark Adaptation Minutes								Brightness Discrimination				
		1	2	3	4	5	6	8	10	13.5 (dark	11.5	9.5	7.5	5.5 light)
Ann Arbor														
Drug	438	6.1	7.1	7.6	8.3	9.0	9.7	10.9	11.5	8.1	7.6	7.5	6.7	5.9
Placebo	422	6.6	7.5	8.0	8.3	8.8	9.1	10.4	10.9	7.4	6.6	6.0	5.2	4.5
												P<.05		
6 Hours, Pikes Peak														
Drug	430	8.3	9.1	10.5	**11.1**	11.7	12.4	12.6	12.6	11.2	8.9	8.7	7.3	6.6
Placebo	417	7.3	8.0	9.3	10.0	10.3	10.7	12.1	12.4	8.5	8.1	7.7	6.5	5.1
						P<.05	P<.01			P<.01				
24 Hours, Pikes Peak														
Drug	480	7.8	9.0	10.1	10.6	10.4	11.0	12.3	12.6	10.8	9.8	9.1	8.1	6.8
Placebo	442	7.5	8.3	9.1	9.8	10.3	10.8	11.9	12.4	10.2	9.5	8.7	7.2	6.2
Day 4, Pikes Peak														
Drug	474	7.9	9.2	10.3	10.8	11.3	11.8	12.4	12.7	10.3	9.4	8.7	7.8	6.9
Placebo	444	6.6	7.7	8.5	9.3	9.8	10.4	11.7	11.9	9.2	8.6	7.7	6.5	5.6
			P<.05	P<.01	P<.05	P<.05	P<.05		P<.05				P<.05	P<.01

generalized decrement in the performance of the high-altitude subjects relative to low-altitude controls but no apparenet differences between placebo- and drug-treated subjects (Table 6). The remaining tests (nonsense syllable memory, Fitts tapping, Stroop color reading) did not reveal differences between the low- and high-altitude or the drug- and placebo-treatment groups (data not shown).

D. DISCUSSION

In this double-blind study, one group of subjects was pretreated with pharmacological agents designed to elevate 2,3-DPG levels and thus, right-shift oxygen dissociation curves prior to ascent to Pikes Peak (4300 m). A second group was given placebos following the same treatment protocol as the drug group. Our results showed that even the minimal increase in 2,3-DPG levels and right-shift of the dissociation curve induced in the drug-treated group was beneficial by some criteria during short-term, high-altitude exposure. Drug-treated subjects felt better, as measured by a symptomatology questionnaire and clinical evaluation, and showed less deterioration of CNS function, as measured by the darkness adaptation visual task. The results of our study did not indicate any benefit for the drug group on any of the cognitive tasks, the critical flicker fusion test. Likewise, ventilation, cardiac output, and alveolar O_2 and CO_2 tensions were the same in the two treatment groups.

The differences between the groups in symptomatology and dark adaptation were statistically significant due to the consistency rather than the magnitude of group differences. The differences in 2,3-DPG levels and curve position while statistically significant were also not of great magnitude. The pharmacologic regimen did not produce as great a rise in 2,3-DPG levels as expected or desired due to the limited effectiveness of phosphate-based regimens. The results of this study suggest that agents with a greater effect on 2,3-DPG levels and oxygen dissociation curve position are deserving of trial for possibly more substantial effects on acute responses to high altitude.

The importance of oxygen dissociation curve position for human high-altitude adaptation has received a variety of interpretations which has suggested benefit from rightward as well as leftward shifts in curve position. Grover et al. (1976) showed that a right-shift permits greater myocardial oxygen extraction at high altitude, thus preventing hypoxia of the heart muscle and permitting the same level of oxygenation without an increase in blood flow. Observations that women have higher 2,3-DPG levels than men and right-shifted curves while having fewer and milder symptoms of AMS compared to men also supports the values of the right-shift. The brain and the heart are similar, in that as long as arterial oxygen saturation remains high, right-shifts will benefit either high or low oxygen demand organs by permitting more oxygen to be extracted or greater oxygen desaturation to occur.

Arguments opposing the benefit of right-shifted dissociation curve at high altitudes have stressed the importance of increased ventilation and blood flow on tissue oxygen availability. Another line of reasoning has suggested that a left-shifted oxygen dissociation curve position is beneficial at high altitude. The llama, indigenous to the Andean altiplano, and the fetus of many mammalisn species, including human beings, both evidence left-shifted oxygen dissociation curves. Rats given cyanate to left-shift their curves showed reduced mortality compared to rats with normal curve position at elevations of 8600 m.

The evaluation of oxygen dissociation curve position in high-altitude adaptation must take several factors into account. The ambient altitude or PO_2 is important because oxygen saturation of the arterial blood is impaired once the arterial PO_2 is in the exponential phase of the sigmoid-shaped dissociation curve. Hence, the fetus in utero or left-shifted rats at 8600 m benefit by being able to achieve higher arterial oxygen saturations than right-shifted animals. Tissue oxygen requirements also must be considered. Tissue of an animal that is native to high altitude such as the llama may be able to extract oxygen at lower PO_2's or enzymes with lower oxygen requirements than non-native animals. Likewise, human embronic and fetal tissues appear not to require as high a PO_2 as the tissue of the adult.

The present study has the advantages of direct comparison within the same species on the effects of change in oxygen dissociation curve position. We have shown that a small right-shift in hemoglobin-oxygen dissociation curve position is associated with some, although significant, improvement in dark adaptation and decreased severity of AMS symptoms. Thus, the present study proves the benefit of a right-shifted dissociation curve during the initial period of exposure to an elevation of 4300 m.

IV. OVERALL SUMMARY

As a result of our work, we believe we have a relatively valid picture of the normal red cell biochemical adjustment to altitude. This adaptation involves primarily the first part of the glycolytic pathway, and involves an activation of both HK and PFK. These activations result in a buildup of 2,3-DPG, and a right-shift of the hemoblobin oxygen dissociation curve.

Second, we believe we have been partially successful in obtaining pre-adaptation to altitude by a pharmacological approach to red cell metabolism. The term "partially" refers to rather modest decrease in oxygen affinity we were able to obtain with available agents, thus preventing a test of whether major performance differences could be obtained. However, even with this modest right-shift of the oxygen dissociation curve, we were able to detect statistically significant effects on CNS performance. This, we think, bodes well for this approach in the future if we can obtain more potent therapeutic agents.

In addition to these two major areas of work, the contract facilitated a good deal of related scientific work in our laboratory (see list of papers).

V. LIST OF PAPERS SUPPORTED BY ARMY CONTRACT DADA17-69-C-9103

Eaton, J. W., Faulkner, J. A., and Brewer, G. J.: Response of the human red cell to muscular activity. Proc. Soc. Exptl. Biol. Med. 132:886-887, 1969.

Brewer, G. J.: Erythrocyte metabolism and function: Hexokinase inhibition by 2,3-diphosphoglycerate and interaction with ATP and MG^{2+}. Biochim. Biophys. Acta 192:157-161, 1969.

Eaton, J. W., Brewer, G. J., Schultz, J. S., and Sing, C. F.: Variation in 2,3-disphosphoglycerate and ATP levels in human erythrocytes and effects on oxygen transport. In: Red cell metabolism and function, G. Brewer (ed.). New York: Plenum Press, pp. 21-38, 1970.

Faulkner, J. A., Brewer, G. J., and Eaton, J. W.: Adaptation of the red blood cell to muscular exercise. In: Red cell metabolism and function, G. Brewer (ed.). New York: Plenum Press, pp. 213-227, 1970.

Brewer, G. J., Eaton, J. W., Weil, J. V., and Grover, R. F.: Studies of red cell glycolysis and interactions with carbon monoxide, smoking, and altitude. In: Red cell metabolism and function, G. Brewer (ed.). New York: Plenum Press, pp. 95-114, 1970.

Brewer, G. J., and Eaton, J. W.: Erythrocyte metabolism: Interaction with oxygen transport. Science 171:1205-1211, 1971.

Brewer, G. J., Oelshlegel, F. J., Jr., and Eaton, J. W.: Biochemical, physiological, and genetic factors in the regulation of mammalian erythrocyte metabolism and DPG levels. In: Oxygen affinity of hemoglobin and red cell acid base status, Alfred Benzon Symposium IV, M. Rørth and P. Astrup (eds.). New York: Academic Press, Inc., pp. 539-551, 1972.

Brewer, G. J.: Clinical implications of variation in erythrocyte oxygen affinity: (A) blood storage and (B) arteriosclerosis. In: Oxygen affinity of hemoglobin and red cell acid base status, Alfred Benzon Symposium IV, M. Rørth and P. Astrup (eds.). New York: Academic Press, Inc., pp. 629-645, 1972.

Brewer, G. J., Oelshlegel, F. J., Jr., Schoomaker, E. B., and Knutsen, C. A.: Potential effects of hemoglobin concentration of red cell metabolism together with observations on red cell metabolic differences between men and women. In: Hemoglobin and red cell structure and function, G. J. Brewer (ed.). New York: Plenum Press, 28:99-119, 1972.

Moore, L. G., Brewer, G. J., and Oelshlegel, F. J., Jr.: Red cell metabolic changes in acute and chronic exposure to high altitude. In: Hemoglobin and red cell structure and function, G. J. Brewer (ed.). New York: Plenum Press, 28:397-413, 1972.

Oelshlegel, F. J., Jr., Brewer, G. J., Penner, J. A., and Shoomaker, E. B.: Enzymatic mechanisms of red cell adaptation to anemia. In: Hemoglobin and red cell structure and function, G. J. Brewer (ed.). New York: Plenum Press, 28:377-396, 1972.

Meyers, N. L., Brewer, G. J., and Oelshlegel, F. J., Jr.: Iron-ATP, a by-product of acid extraction of whole blood or red blood cells. Biochim. Biophys. Acta 320:397-405, 1973.

Moore, L. G., Brewer, G. J., Oelshlegel, F. J., Jr., Brewer, L. F. and Shoomaker, E. G.: Pharmacologic stimulation of erythrocyte 2,3-diphosphoglycerate production in vivo. J. Pharm. Exptl. Therap. 203:722-728, 1977.

Moore, L. G., Brewer, G. J., Oelshlegel, F. J., Jr., and Rose, A. M.: Pharmacological stimulation of red blood cell metabolism for high altitude preadaptation. In: Oxygen transport to tissue, D. F. Bruley and H. I. Bicher (eds.). New York: Plenum Publishing Corp., 693-698, 1973.

Brewer, G. J., Sing, C. F., Eaton, J. W., Weil, J. V., Brewer, L. F., and Grover, R. F.: Effects on hemoglobin oxygen affinity of smoking in residents of intermediate altitude. J. Lab. Clin. Med. 84:191-205, 1974.

Brewer, G. J.: General red cell metabolism. In: The red blood cell, 2nd ed., D. Mac N. Surgenor (ed.). New York: Academic Press, Inc., 1:387-433, 1974.

Eaton, J. W., and Brewer, G. J.: Pentose phosphate metabolism. In: The red blood cell, 2nd ed., D. Mac N. Surgenor (ed.). New York: Academic Press, Inc., 1:435-471, 1974.

VI. REFERENCES

Chance, B., Holmes, W. F., Higgins, J., and Connelly, C. M.: Nature 182:1190, 1958.

Ferguson, R. Faulkner, J., Julius, S., and Conway, J.: Comparison if cardiac output determined by CO_2 rebreathing and dry-dilution methods. J. Appl. Physiol. 25:450-454, 1968.

Grover, R. F., Lufschanowski, R., and Alexander, J. K.: Alterations in the coronary circulation of man following ascent to 3100 m altitude. J. Appl. Physiol. 41:832-838, 1976.

Minakami, S., Suzuki, C., Saito, T., and Yoshikawa, H.: Studies on erythrocyte glycolysis. I. Determination of the glycolytic intermediates in human erythrocytes. J. Biochem. 58:543, 1965.

Moore, L. G., Brewer, G. J., Oelshlegel, F. J., Jr., Brewer, L. F., and Shoomaker, E. B.: Pharmacologic stimulation of erythrocyte 2,3-diphosphoglycerate production in vivo. J. Pharm. Exptl. Therap. 203:722-728, 1977.

Oelshlegel, F. J., Jr., Brewer, G. J., Penner, J. A., and Shoomaker, E. B.: Enzymatic mechanisms of red cell adaptation to anemia. In: Hemoglobin and red cell structure and function, G. J. Brewer (ed.). New York: Plenum Press, 28:377-396, 1972.

Rørth, M., and Nygaard, S. F.: Phosphate metabolism of the red cell during exposure to high altitude (4500 m). In: Oxygen affinity of hemoglobin and red cell acid base status, Alfred Benzon Symposium IV, P. Astrup and M. Rørth (eds.). New York: Academic Press, Inc., pp. 599-608, 1972.

Rose, A. M.: Doctoral Dissertation, Department of Psychology, The University of Michigan, Ann Arbor, Michigan, 1973.

Simonsen, E., and Brozek, J.: Flicker fusion frequency. Physiol. Reviews 32:349-378, 1952.

LIST OF PERSONNEL RECEIVING CONTRACT SUPPORT

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John Eaton, Ph.D.
Carl Coan, M.S.
Eric Schoomaker, M.D./Ph.D.

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